DESIGNWORKS

BERKELEY LAB

WRAP-UP REPORT THERMAL CYCLER PROJECT

Client: DOE Joint Genome Institute

Instrumentation Program

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PROJECT SUMMARY - KEY ISSUES

The JGI thermal cycler project began 4/30/01 and ended 06/15/01.

JGI thermal cycled biological test samples showed signs of evaporation around the sides of microplate test samples, causing lost data. A second order issue was that lab technicians were frustrated with the thermal cycler biological test process. They were tightening the bonnet of the thermal cycler without instrument feedback indicating when the seal was tight. Repetition of this process wore their fingers raw.

JGI requested that DesignWorks confirm the evaporation and then identify the cause of the evaporation and based on that determination assess possible solutions. If practical, the solution would address the second order issues.

Pressure tests were performed using pressure sensitive carbon paper. The tests indicated that at very low pressures there was leakage between the platen and the microplate. However, above a certain PSI, the results showed that there was fairly uniform pressure. DesignWorks analyzed the test results in detail and also determined that the uniformity of the seal between the platen and the microplate was dependent on the batch of microplates. Test results varied greatly between microplate types.

DesignWorks presented findings to JGI and recommended possible options. (See sections JGI MICRO Pressure Test Report and What's Next? for more detail).

JGI chose to perform pressure tests using Pressurex Micro rated carbon paper on each batch of microplates. This would give them immediate feedback regarding the sealing properties of a specific plate. In addition, technicians will modify the test procedure by consistently setting the thumbscrew pressure as high as possible.

THERMAL CYCLER PROJECT PLAN

Project Description: JGI requested that DesignWorks modify an instrument that is used for thermal cycling of biology samples. The instrument does not equally seal across the top plate. Our goal is to modify the instrument so that it seals better as well as providing a psi readout so that all instruments are calibrated equally. An added bonus to JGI would be to modify the instrument in such as way that lab technicians don't have to continuously adjust the pressure dial. This is inconvenient and wears on fingers.

Scope: Initially one instrument will be modified and then the remaining instruments will be modified the same way. It is estimated that there are a minimum of 64 instruments that will need modification.

DesignWorks Process: We see the project going through all the stages of a "Gadget" project. For this project, the stages include the following design tasks:

- 1. Specify the design problem
- 2. Gain an understanding of relevant issues (how it is used, desired life, conditions used, who will use it, etc.)
- 3. Perform concept generation to identify a variety of concepts
- 4. Define constraints, requirements, desires, and prioritize values (e.g. do you want a low-cost fix that wears out, is it important for the solution to be easy to use?)
- 5. Narrow concepts to a small number for further study and detailing
- 6. Identify critical components of concept(s) (e.g. does the concept depend on strain gages? Does it depend on an operator who is well trained?)
- 7. Validate critical components through prototyping, testing, or analysis (if we go with a strain-gage concept, we'll make a prototype with gages mounted on it)
- 8. Incorporate changes, tweaks, and other details into final design (this task and numbers 6 and 7 will go through iterations)
- 9. Procure and fabricate necessary items
- 10. Assemble and test final product
- 11. Write a "How to Use" document to deliver with the product
- 12. Deliver and train users on its use

FIRST MEETING SUMMARY 4/30/01

Marty Pollard, Ken Chow and Lisa Gullo met to discuss the Thermal Cycler Project Plan and the MJ thermal-cycler instrument that DesignWorks will be modifying for JGI.

KEY POINTS

Requirement: DesignWorks shall design a device to calibrate pressure for the MJ thermal cycler's platen seal. The device shall have read-out instrumentation and the seal shall be measured evenly (in approximately five different places).

Scope: DesignWorks first order goal is to design a calibration device. After developing the calibration device, the scope of the project may extend to the task of developing a plate that seals better as well as modifying the instrument in a way that the dial didn't wear on fingers. The modifications may be applied to roughly sixty MJ instruments.

Instrument Operation: The instrument is cycled approximately 20 to thirty times per use and the temperature varies from 65° - 95° c. The plastic sampler is inserted once at room temperature.

Summary: In response to some key questions regarding the project, Marty Pollard stressed that his highest priority is to develop a calibration device. Concept generation should be a part of the DesignWorks process, but only a small percentage our time should be dedicated to this. Marty will be the primary contact, however in a couple of week's time, Karl Petermann may be available as a main contact. In the meantime, questions will be directed toward Marty. Marty provided a charge number, LWRAES, for parts, etc. Marty will be bringing DW another plate for DW to analyze and test. People who may be available to help on this project are Charlie Reiter, electronics assembly and test, B25, and Mario Cepeda, machining, B25.

BRAINSTORMING SESSION RESULTS

CONCEPT SUMMARY FOR CONCEPT 1: STRAIN GAGE SYSTEM

DESCRIPTION OF CONCEPT

A calibration plate is used to provide data on pressure applied by the thermal cycler lid. Strain gages mounted at strategic locations on the lid are wired to a cable that connects to a meter that displays the results.

Reliability: Fairly reliable. Strain gages are very mature technology and issues such as drift should not be a problem after the system is debugged and if the system is used properly.

Accuracy: Highly accurate. Strain gage design must be properly conducted (placement, orientation, configuration). Strain readings must be carefully calibrated to provide accurate pressure values.

Ruggedness: Medium. Several items need to be handled properly (and inspected) to avoid misreads due to damage. These include strain gages, leads, and electronic components.

Ease of use: Medium. Proper alignment is necessary. Features can be added to improve ease of use (conversion of voltages to pressure, strain relief, alignment pins, etc.)

Area coverage: Minimal. One gage needed for each location. Reasonable number of gages is no more than six, therefore we would only get readings at these six points.

Feedback time: Instantaneous. Readings can be displayed continuously as the pressure is adjusted.

Development cost: Medium. Effort includes: analysis, detail design, selecting components, design of readout subsystem, and assembly of system components. Individual components are inexpensive but putting a system together that works takes development effort.

Development time: Medium. Associated with development time.

Advanced functions: None.

Long-term costs: Very low. No single-use items are needed to perform a reading.

Versatility: Limited. System will probably need to be adapted for other applications.

Overall this concept will likely lead to a device that provides a reading that can be correlated to pressure, but a lot of effort is needed to ensure accuracy, reliability, ruggedness, and ease of use. The effort may include FEA to determine strain gage location and configuration. Testing is likely needed to calibrate readings to pressure.

The main drawbacks to this concept are the limited number of pressure points and the amount of development needed.

The main advantages to this concept are the use of mature technologies and the very low long-term costs.

CONCEPT SUMMARY FOR CONCEPT 2: PRESSURE SENSITIVE FILM SYSTEM

DESCRIPTION OF CONCEPT

Pressure sensitive film is inserted between the platen and the microplate. The film is removed and scanned to determine pressure distribution

Reliability: Very reliable. Pressure sensitive film has been used in many industries and is a mature product. The simplicity of the system leads to high reliability. Film should not be used beyond its shelf life.

Accuracy: Indeterminate. Manufacturer of film states 10% accuracy on readings. However, ultra-low pressure levels (less than 20 psi) have low accuracies and can only provide relative measurements.

Ruggedness: Very rugged. System has few parts and film can be handled like normal paper.

Ease of use: Easy to use. Proper alignment is not necessary. Although system is easy to use, proper interpretation of results is important for accuracy.

Area coverage: Maximum. A complete pressure profile across all mating surfaces is provided.

Feedback time: Delayed. Information on pressure is obtained only after film is removed and scanned.

Development cost: Low. Information is available with minimal development effort. Effort is required to

determine proper grade of film.

Development time: Low.

Advanced functions: None.

Long-term costs: Medium. One sheet of pressure sensitive film for testing costs up to \$5.

Versatility: Medium. System can be used with different microplates.

Overall, this concept provides a very fast path to obtaining pressure readings. However, the possibly low values may ultimately make this concept non-viable. Very little up-front investment is needed.

The main drawbacks to this concept are its long-term costs and difficulties in using the system for the desired low-pressure ranges.

The main advantages to this concept are its simplicity, low up-front costs, and complete coverage of the pressure surfaces.

RESOURCES FOR PRESSURE SENSITIVE FILM

http://www.fujiprescale.com/html.online store.htm

CONCEPT SUMMARY FOR CONCEPT 3: TEKSCAN FORCE & PRESSURE SENSORS

DESCRIPTION OF CONCEPT

Tekscan Pressure Sensors: Button shaped single sensors are placed strategically along the platen to measure the resistance. A small circuit measures the output voltage that is routed to a data acquisition box that plugs into the serial port of a computer. Readings are displayed in pounds.

Reliability: Medium to high reliability. This is a mature product.

Accuracy: Highly accurate. Accuracy range is +/- 2 to 10%

Ruggedness: Medium. This system has several items which must be handled carefully in order to avoid

misreads.

Ease of use: Intermediate. After the initial set up, the easy of use is relatively easy.

Area coverage: Medium. The entire platen cannot be covered at one time.

Feedback time: Instantaneous. Results can be read on a computer desktop.

Development cost: Medium. The effort includes analysis of results, design of circuits and installation of software package.

Development time: Medium. Circuits must be designed for each sensor that is used.

Advanced functions: None.

Long-term costs: Very low. The sensors can be used over again for different tests.

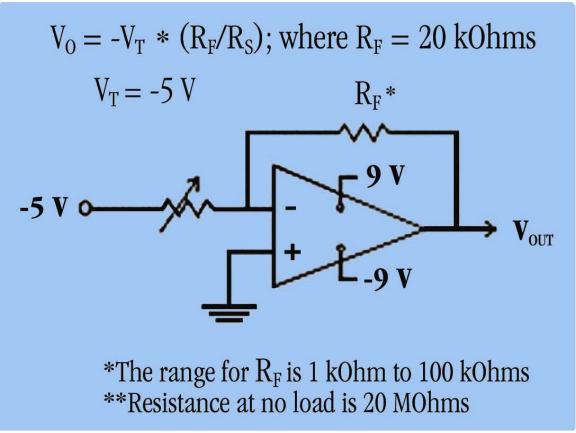
Versatility: Medium to High. This system can be used with different microplates.

This concept will provide different readings for each sensor. The values are in pounds and analysis will be interpolated to determine the force for the entire surface of the platen. The test set up requires that different circuits be designed for each sensor. Calibration is required to determine the accuracy of the test.

The main drawback is that it is limited to a number of pressure points and some development time is required.

The main advantage is that this test is accurate, versatile, and reliable.

CIRCUIT DIAGRAM FOR PRESSURE SENSORS



RESOURCES FOR SENSOR VENDORS

http://www.tekscan.com/

http://www.endevco.com/

http://www.endevco.com/monitoring/monitoring main.htm

CLIENT BRAINSTORMING FOLLOW-UP MEETING - SUMMARY

May 4, 2001

Attendance: Ken Chow, Marty Pollard, Lisa Gullo

I showed Marty our concept summaries for the strain gage concept and the pressure sensitive film concept. We talked about how the users actually set the pressure. Marty says they probably do both (turn the knob the extra 3/4 turn after opening the top as well as turning the knob an extra 3/4 turn while it's closed).

I reviewed our initial understanding of the 4-bar hinge linkage and how it pertains to setting the pressure. I indicated that the pressure is generated by the compliance between two points of the 4-bar linkage system. Marty feels the compliance is in the bonnet lid. I pointed out how the older 96 well unit has its "fixed" linkage bar poorly secured and how the new unit has the same bar rigidly attached. Wear and tear may result in loosening of this "fixed" linkage and therefore effect the pressure applied to the microplate. Marty says when he gets back to JGI he will examine the units to check for looseness of the "fixed" linkage.

We tried to imprint the "Low" pressure grade film with the 384 well thermal cycler unit, but it made no impressions.

I reviewed information gathered on the pressure sensitive film concept: cost of film, how it works. We briefly discussed the disadvantage of long-term costs associated with this concept. Marty says it is expensive but could be tolerable if it is the best solution.

Meeting action items and strategy:

Since the pressure sensitive film requires no development effort, we will pursue the following plan:

- 1. Purchase small quantities of the pressure sensitive paper (micro, ultra-low, and super-low grades)
- 2. Concurrent to #1, plan and conduct an initial process for image analysis of imprinted film. The process includes creating known imprints, scanning an image of the imprinted film, and performing simple image analysis to correlate percent color saturation with pressure.
- 3. Test out the pressure sensitive film concept on several thermal cycler units at JGI. This test will also be used to gather preliminary data.

4. Analyze data to determine further course of action (further pursuing pressure sensitive paper concept, pursuing strain gage concept, testing to gather more information, etc.)

TEST PLAN TO CALIBRATE PRESSURE INDICATING FILM

One test was completed for the MICRO paper. The results are included in this test plan procedure.

1) Tests will be performed on three classification ratings of Pressure Indicating Film. These classifications are:

Rating	MICRO	ULTRA LOW	SUPER LOW
Pressure Range	2-28 psi	28-85 psi	70-350 psi

- The test runs will be performed using either weights or a load frame. The test method for the Pressure Indicating Film rated Micro will use weights. The test methods for the film rated Ultra Low and Super Low will use the load frame. Tests will be performed in the following manner. A "stamp" will make an impression into the film. Pressure is added using either weight or a load frame. The surface area of the stamp is measured and calculated. Multiply the target pressure by the stamp's surface area to determine the load to apply.
- 3) Stamp size (steel): length = 1 inch x width = 1 inch = Area 1 inch 2
- 4) Each classification of Pressure Indicating Film will be tested for a series of pressures. They are the following:

MICRO

Target	Target	Run 1 Applied Force	Run 2 Applied Force	Run 3 Applied Force
Pressure	Force			
1 psi	1 psi	1.01 psi	1.01 psi	.98 psi
2 psi	2 psi	2.0 psi	2.05 psi	2.01 psi
7 psi	7 psi	6.95 psi	7.05 psi	7.01 psi
12 psi	12 psi	12.03 psi	12.03 psi	12.01 psi
18 psi	18 psi	18.13 psi	18.11 psi	18.13 psi
24 psi	24 psi	23.98 psi	24.05 psi	24.05 psi

ULTRA LOW

Target	Target	Run 1 Applied Force	Run 2 Applied Force	Run 3 Applied Force
Pressure	Force			
18 psi				
28 psi				
44 psi				
60 psi				
85 psi				
95 psi				

SUPER LOW

Target	Target	Run 1 Applied Force	Run 2 Applied Force	Run 3 Applied Force
Pressure	Force			
50 psi				
70 psi				
150 psi				
200 psi				
350 psi				
400 psi				

- On the Pressure Indicating Film, document each test run. Documentation will include the following: 1) the test run numbers 2) the time and dates 3) the test taker's signature.
- 6) Scan the Pressure Indicating Film test results into a computer
- Using an image processing software, convert the film paper's dot image results to a grayscale digital image. Average the saturation density by looking at a given pixel area, i.e. 100 pixels by 100 pixels. This area will map to the film paper's test impression. Analyze the percentage of saturation for each run.
- 8) Average the saturation density for each test run. Record values on a pressure and saturation table like the one below.

	SATURATION %		
Pressure	Run 1	Run 2	Run 3
i.e., 50 psi			

- 9) Plot the results on a graph. Pressure will be on the "x" axis and saturation on the "y" axis. Add a "best fit" curve to the graph.
- Analyze the graph to determine the preciseness of the film paper's ability to measure pressure.

CALCULATION OF MICROPLATE SEALING SURFACE AREA

	384 plate	96 plate
OD sample wells	4.09 mm	6.81 mm
ID sample wells	3.1 mm	5.29 mm
Thickness	.99 mm	1.52 mm
Outer Area	13.13822 mm^2	36.4237 mm^2
Inner Area	7.547676 mm^2	21.97866 mm^2
# of units (or sample wells)	384	96
Total Area	2146.768 mm^2	1386.724 mm^2
Total Area	3.327498 in^2	2.149427 in^2

JGI MICRO PRESSURE TEST REPORT

Monday, June 4, 2001, Ken Chow and I went to the Joint Genome Institute (JGI) in Walnut Creek to perform pressure tests on a sample of the thermal cycler model MJ-384 instruments. The tests were performed using Pressurex Micro pressure paper, a type of carbon pressure paper that measures a pressure range of two to twenty-eight pounds per square inch (psi). The goal of the test was to identify if there are areas that do not seal at a minimum of two psi of pressure and to identify the pressure pattern between the platen seal and microplate. Our test confirms that in many cases the platen and microplate do not completely seal across the surface and that there are repeatable patterns. (These results are discussed further in the conclusions.) We performed our tests using seven different thermal cycler units and four different microplate inserts. Twenty-four tests were completed. Below are the some conclusions that we reached based on our tests.

Conclusion Summary:

- 1. Measurements are highly repeatable
- 2. Pressure variation is primarily due to different microplates
- 3. Poor sealing occurs on the left and right sides of the microplates
- 4. The DesignWorks microplate seals more uniformly
- 5. The Greiner microplate seals poorly in the center.
- 6. Adding more turns to the thumbwheel screw improves the seal, but doesn't completely seal the surface
- 7. Thicker paper does not change our results

CONCLUSION 1: <u>MEASUREMENTS ARE HIGHLY REPEATABLE</u> (we can draw conclusions from one measurement reading because if we take multiple readings with everythingthe same, we will get the same pressure signatures).

This conclusion is drawn from three sets of data:

- 1. Samples #2-5 are from the same MJ thermal cycler and the same microplate. The pressure signatures are very similar. This implies that when everything is the same, the pressure signatures are repeatable.
- 2. Samples #6 and #9 are identical except that in one we turned the adjustment wheel before closing the lid and in the other we turned the wheel after the lid was
- 3. closed. The pressure signatures are very similar. These two reinforce our assertion that the repeatability of the measurement is very good.
- 4. Samples #23 and 24 are very similar. Even though we were confused by the smudginess of #23, sample #24 shows that repeating the measurement gave the same
- 5. results.
- 6. Samples #10-21 were taken using the same microplate, unlabeled #2. The results indicate that regardless of slight changes in the test procedure, there is a consistent pressure sample signature.

CONCLUSION 2: <u>PRESSURE VARIATION IS PRIMARILY DUE TO</u> DIFFERENT MICROPLATES.

This conclusion is drawn from the following sets of data and previous conclusion:

- 1. Samples #5-8 are from the same MJ thermal cycler but four different microplates. The pressure variation in them is very different.
- 2. Samples #15-19 are from different MJ thermal cyclers but the same microplate. The pressure variation in them is very similar. This implies that different thermal cyclers produce the same pressure signature as long as the same microplate is used.
- 3. Measurements are repeatable (see conclusion 1 above). This is necessary to address the criticism that the differences in Samples #5-8 are merely due to noise
- 4. variations. If our measurements were not repeatable, we would not be able to draw conclusion two only from item #1 above.
- 5. Samples #2-11 were taken with the same thermal cycler unit, MJ384-79, but different microplates. The results are varied. Test samples #2-5 and test samples #10-12 use the same microplate, the DesignWorks plate and the unlabeled 2 plate, respectively. Test samples #6, #9, #22-24, the pressure patterns are very similar. These tests used the same microplate, Greiner, but two different units were tested. In tests where the same microplate was used, the sample signatures are most similar.

CONCLUSION 3: <u>POOR SEALING OCCURS ON THE LEFT AND RIGHT SIDES OF THE DIFFERENT MICROPLATES</u>.

This conclusion is drawn from the following sets of data:

- 1. Samples #1-21, and #23-24 all have a similar pressure signature indicating an incomplete seal on the left side of the microplate (sample 20 is flipped but shows the same results).
- 2. Samples #1-5, #7-8, and #10-21 all indicate poor sealing on the right side of the microplate (sample 20 is flipped).
- 3. Samples #10-11, and #23-24 were performed with slight procedural change. The number of thumbwheel turns was increased past nominal pressure. In all of these cases, the pressure pattern indicates that there is an incomplete seal due to some of the circles not being completely filled.
- 4. Samples #1-9 followed a procedure of leaving the top of the thermal cycler open and then adjusting the thumbwheel screw. Sample 10 varied this procedure by tightening the thumbwheel screw after the top of the thermal cycler was closed. The results indicate that this variation in procedure doesn't change the pressure pattern.

CONCLUSION 4: THE DESIGNWORKS MICROPLATE SEALS MORE UNIFORMLY.

This conclusion is drawn from the following sets of data:

- 1. Samples #6, #7, and #8 represent tests taken with the Greiner, unlabeled 1, and unlabeled 2 microplates. These tests all reveal a similar pattern even though different microplates were used. The patterns show incomplete sealing along either the left or right side, or the center.
- 2. Samples # 1-5 indicate that the pressure pattern is fairly consistent. These tests were all used with the DesignWorks microplate. Sealing patterns are very consistent with this plate.

CONCLUSION 5: THE GREINER MICROPLATE SEALS POORLY IN THE CENTER.

This conclusion is drawn from the following sets of data:

1. Samples #6, #9, and #22-24 all show incomplete circles in the center indicating that there is incomplete sealing in this location. This is true even in samples #23 and #24 which increase the pressure by one half turn.

CONCLUSION 6: <u>ADDING MORE TURNS TO THE THUMBWHEEL SCREW</u> <u>IMPROVES THE SEAL, BUT DOESN'T COMPLETELY SEAL THE SURFACE</u>

1. Samples #10 and #11 show that the seal improves when pressure is increased, however there is still an incomplete seal around the sides. Samples #15-24 confirm the same results.

CONCLUSION 7: THICKER PAPER DOES NOT CHANGE OUR RESULTS

1. Samples #4, #5, #21, and #24 were all taken with thicker paper. These results do not vary from other similar samples that were taken using either the same microplate or the same unit number.

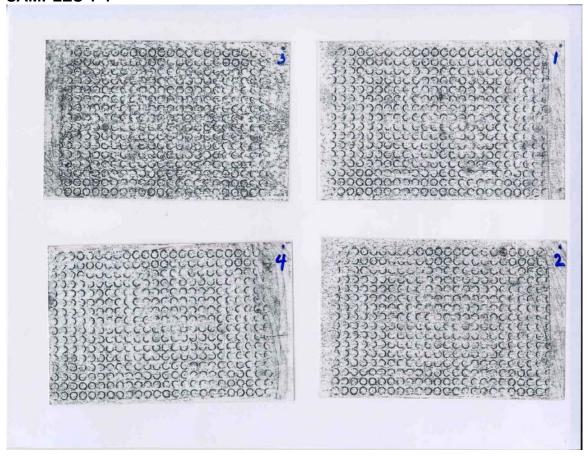
THERMAL CYCLER TEST PROCEDURE

- 1. Prepare samples, cut sample size slightly larger than microplate, designate a "peeling side", make this side longer
- 2. Open thermal cycler
- 3. Load microplate (numbers and letters read right side up)
- 4. Place carbon paper facing downward
- 5. Turn blue thumbwheel counterclockwise to the lowest pressure
- 6. Latch
- 7. Tighten counter-clockwise until pressure is felt
- 8. Open lid
- 9. Turn ³/₄ turn
- 10. Add paper, wait ten seconds
- 11. Remove paper
- 12. Carefully peel away carbon paper and place plastic coating over impression

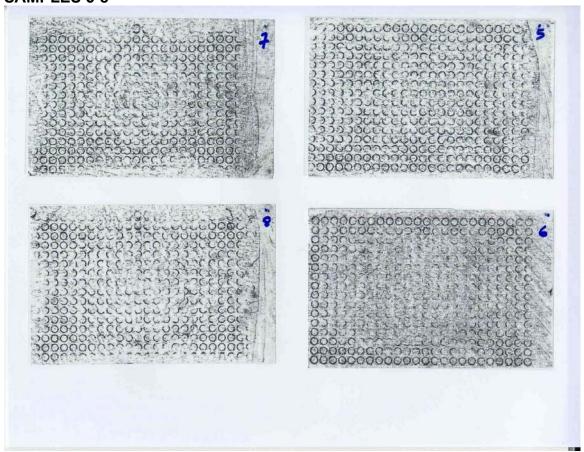
THERMAL CYCLER TEST CONDITIONS

Sample	Unit	Microplate	Microplate	Pressure	Comments
Number	Number	Number	Orientation		
1	384-80	DesignWorks	Normal	Nominal	Lid left open when turning plate 3/4 turn
	384-79	DesignWorks	Normal	Nominal	Lid left open when turning plate 3/4 turn
	384-79	DesignWorks	Normal	Nominal	Pressurex paper offset to the left
4	384-79	DesignWorks	Normal	Nominal	Backing paper not removed
	384-79	DesignWorks	Normal	Nominal	Backing paper not removed: Baseline
	384-79	Greiner	Normal	Nominal	
	384-79	Unlabeled #1	Normal	Nominal	
8	384-79	Unlabeled #2	Normal	Nominal	
9	384-79	Greiner	Normal	Nominal	Lid closed first and then turned thumbwheel 3/4 inch
10	384-79	Unlabeled #2	Normal	Nominal + 1 turn	Added full turn
11	384-79	Unlabeled #2	Normal	Nominal + 1 1/2 turn	Lid didn't close completely
12	384-80	Unlabeled #2	Normal	Nominal	
13	384-78	Unlabeled #2	Normal	Nominal	
	384-77	Unlabeled # 2	Normal	Nominal	
	384-76	Unlabeled #2	Normal	Nominal + 1/2 turn	
	384-75	Unlabeled #2	Normal	Nominal + 1/2 turn	
	384-73	Unlabeled #2	Normal	Nominal + 1/2 turn	
18	384-74	Unlabeled #2	Normal	Nominal + 1/2 turn	
	384-84	Unlabeled #2	Normal	Nominal + 1/2 turn	Carbon paper creased
20	384-73	Unlabeled #2	Upside down	Nominal + 1/2 turn	
21	384-73	Unlabeled #2	Normal	Nominal + 1/2 turn	Pressurex paper turned upside down; backing paper not removed
22	384-73	Greiner	Normal	Nominal + 1/2 turn	
23	384-73	Greiner	Normal	Nominal + 1 turn	Backing paper not removed; Smudging may be result of platen sliding
	384-74	Greiner	Normal	Nominal + 1 turn	

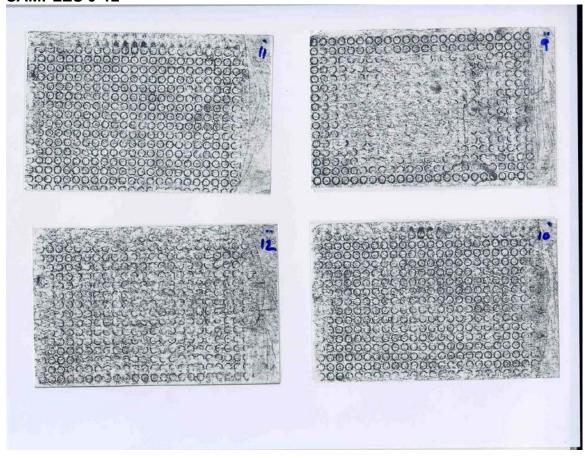
TEST SAMPLES SAMPLES 1-4



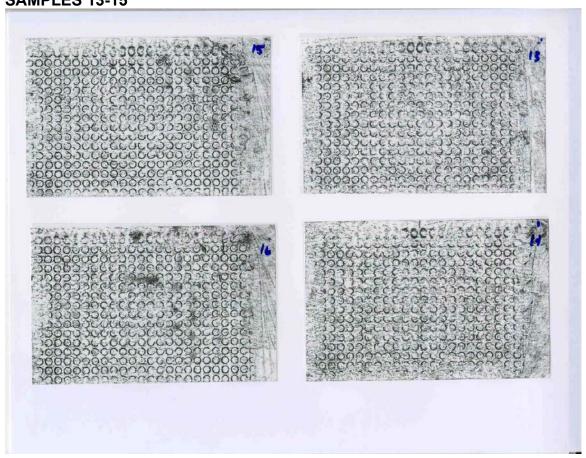
SAMPLES 5-8



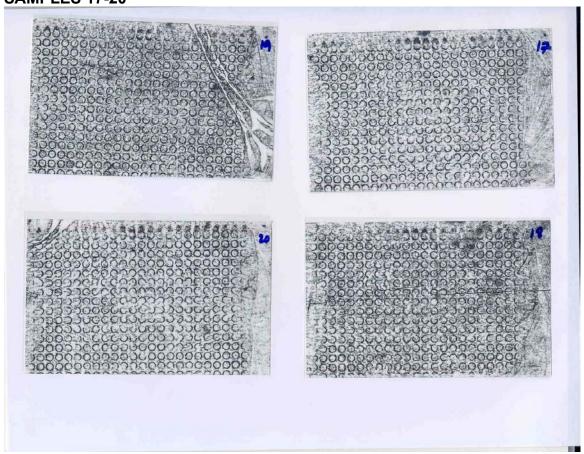
SAMPLES 9-12



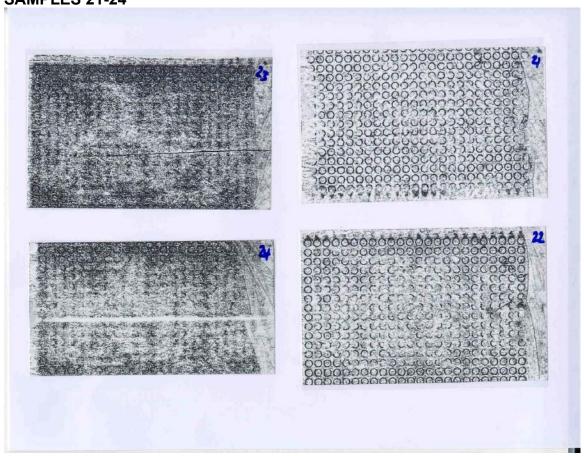
SAMPLES 13-15



SAMPLES 17-20



SAMPLES 21-24



INVENTORY

DesignWorks has the following remaining tools in inventory:

Item	Quantity
Thermal cycler	1
Thermal cycler operations manual	1
Thermal cycler service manual; Chapter 1	1
Microplate	1
Paintbrush	1
Pressurex Micro carbon paper	1 sheet
Fuji Prescale film: Ultra low: 28-85 psi	4 sheets 1 foot long
Fuji Prescale film: Super-low: 70-350 psi	4 sheets 1 foot long
Tekscan pressure sensors	4
Female adaptors	4

WHAT'S NEXT?

- 1) Improve procedure by increasing the size of the sample Pressurex paper so that there is a longer peeling edge to minimize sample smearing.
- 2) Determine how many types of Microplate batches are used. Is there variation amongst each of those batches of microplates?
- 3) Determine how the microplates are used. Are the microplates discarded or reused?
- 4) Provide recommendation to engineers and technicians to set the thumbscrew pressure as high as possible.
- 5) Determine the effect of the sealing film. What kind of material is it made out of? How is it applied and removed?
- 6) Further tests with the possible following modifications:
 - a. peal off rubbery silicone backing
 - b. determine the grade of silicone that we are currently using, replace silicone bonnet lid with different grades of silicone
 - c. add additional layer of silicone to the platen
 - d. test all of the representative batches of microplates
 - e. test individual microplates within each batch to see if there is variation
- 7) Identify goals:
 - a. Do we want to calibrate microplates or thermal cyclers or both?
 - b. Calibrating the microplates will not be effective if they are discarded and/or changed frequently

- c. Calibrating individual thermal cyclers may not be effective if the problem is with the microplates
- d. Do we want to retrofit the bonnet of the thermal cycler to improve pressure?

FOLLOW UP LETTER

June 15, 2001

Marty,

Enclosed is the Pressurex Micro carbon paper along with some of the Microfilm paper. I've also included the manufacturer directions and a paintbrush to use for application. There is some remaining higher rated microfilm paper. If you'd like us to ship you the remainder, we will do so.

Here is the contact information for the vendor:

Sensor Products, Inc. 188 Route 10, Dept 307 East Hanover, NJ 07936-2108

Phone: (973) 884-1755 Fax: (973) 884-1699

This order was placed under: Procard P.O. # 99477 Web Job Order # UC0645

Please let me know if you have any questions.

Regards,

Lisa Gullo (510) 486-4006